Liquid Culture Re-Amplification Protocol by Chong Park

For sublibrary amplification:

Use NEB DH5a High Efficiency (>3 x 10^9)

1) Mix: 10-50 ng of DNA + 100 uL DH5α high efficiency (3x109 cfu/ug) (1 rxn per sublibrary)
2) Transform using the manufacturer's suggested protocol, making sure to perform the full 30min ice-incubation.
3) Recover in 1 mL total with SOC for 1 hour while shaking at 37C.

4a) Take 5ul of recovery. Make serial dilutions and plate them onto LB/Amp plate to calculate transformation efficiency.
4b) Add the rest of recovery to 500 mL LB+Carb. Grow O/N while shaking @ 37°C (16h)

5) Calculate transformation efficiency next day. If the efficiency is higher than ~200 colonies per sgRNA construct in the library, harvest cells and purify the library.
6) Depending on your pellet weight use multiple Maxiprep columns (Sigma or Zymogen), a Megaprep (Qiagen or Sigma), or Gigaprep (Qiagen, Sigma, Zymogen).
7) Expect a yield ~2mg.

For pooled library

Use MegaX DH10B (>3 x 1010 cfu/µg) electrocompetent cells (Fisher cat# C640003) to achieve higher coverage (~1000 colonies/sgRNA).

*Pre-chill cuvettes on ice before starting
1) Mix 100ng of pooled library with 50ul of MegaX cells.
2) Transform using the manufacturer's suggested protocol (2.0kV, 200 ohms, 25uF in 0.1cm cuvette). Do not ice incubate.
3) Recover in 1mL total with SOC for 1.5-2 hours while shaking at 37C
4a) Take 5ul of recovery. Make serial dilutions and plate with beads to calculate transformation efficiency.
4b) Add the rest of recovery to 500 mL LB+Carb. Grown O/N while shaking @ 37C (16h)

5) Calculate transformation efficiency next day. If the efficiency is higher than 1000 colonies per sgRNA construct in the library, harvest cells and purify the library

6) Depending on your pellet weight use multiple Maxiprep columns (Sigma or Zymogen), a Megaprep (Qiagen or Sigma), or Gigaprep (Qiagen, Sigma, Zymogen).
7) Expect a yield ~2mg.

Illumina sequencing

We strongly recommend deep sequencing the amplified libraries before use.
Follow protocol "Illumina sequencing for amplified library" for optimized protocol on PCR-amplifying the sgRNA region and purification.